

Supplementary Material

Supplementary table S1. Main clinical characteristics of enrolled patients.

ID: identifier; e': mitral annular peak early diastolic velocity; E: peak velocity of mitral inflow during early diastole; TR: Tricuspid regurgitation; PAPS: Pulmonary artery systolic pressure; LAVI: Left atrial volume index; LVEF: Left ventricular ejection fraction; LVMI: Left ventricular mass index; NYHA: New York Heart Association; hs TNI: high sensitivity Troponin I; BNP: Brain Natriuretic Peptide; n.a.: not available.

Patient ID	H2	H3	H4	H5	H6	H7	H8
Age	71	60	66	77	56	75	75
Sex	F	F	F	F	M	F	M
Aortic valve stenosis	yes	yes	yes	yes	yes	yes	yes
Diabetes	no	no	no	no	no	no	no
Hypertension	yes	no	yes	yes	yes	yes	yes
Obesity	no	no	no	yes	no	yes	no
Atrial Fibrillation	no	no	yes	yes	no	no	yes
LAVI (ml/m²)	44	68	29	60	38	54	34
LVMI (g/m²)	188	169	234	119	110	149	108
e' (cm/s)	n.a.	n.a.	n.a.	n.a.	6	5	5
E/e' ratio	19	16	16	16	15	20	16
TR peak velocity (m/s)	3	3.3	2.9	3	2.9	3	3
PAPS (mmHg)	38	25	n.a.	n.a.	31	42	42
LVEF (%)	66	64	74	64	56	76	57
NYHA class	1	2	3	3	3	3	2
Breathlessness	no	yes	yes	yes	yes	yes	yes
hs TNI (ng/l)	7562.9	669.0	9608.3	1761.2	30619.2	13.6	n.a.
BNP (pg/ml)	n.a.	420	866	244	624	156	83

Supplementary Table S2. Antibody list.

Protein	Antibody	Host	Company	Dilution
MDA	ab6463	Rabbit	Abcam	IF 1:2500
CD44	ab119335	Rat	Abcam	IF: 1:50
cTNT	MA512960	Mouse IgG1	Thermo fisher scientific	IF 1:200
γ H2AX	ab11174	Rabbit	Abcam	IF 1:500 WB 1:1000
pCHK1	#2344	Rabbit	Cell Signaling	WB 1:100
pCHK2	#2661	Rabbit	Cell Signaling	WB 1:1000
GAPDH	sc-25778	Rabbit	Santa Cruz	WB 1:1000
TGF β	ab64715	Mouse IgG1	Abcam	WB: 1:1000
COL1A1	#84336	Rabbit	Cell Signaling	WB: 1:1000
α SMA	A 2547	Mouse IgG2a	Sigma-Aldrich	WB: 1:1000

Supplementary Table S3. Primer sequences 5' - 3'.

Gene	Forward primer	Reverse primer
<i>IL1β</i>	CAAAATACCTGTGGCCTTGG	ACTGGGCAGACTCAAATTCC
<i>IL6</i>	ACAAAAGTCCTGATCCAGTTCC	GACTGCAGGAACTCCTAAAGC
<i>TNFα</i>	AGCCCATGTTGTAGCAAACC	AGGACCTGGGAGTAGATGAGG
<i>NF-kB</i>	ACATCTCCTGCTTAGTG	TCACATCTGGTTGATTT
<i>TGFβ1</i>	AAGTGGACATCAACGGGTTC	GTCCTTGCGGAAGTCAATGT
<i>GAPDH</i>	ATGTTCGTCATGGGTGTGAA	GTCTTCTGGGTGGCAGTGAT

Supplementary table S4. Cytokines released in the culture supernatant by control C-MSC in static or dynamic culture conditions (n=6 each).

	Non-stretched	Stretched	P value
Adiponectin (pg/ml)	5019 ± 1618	4884 ± 836.2	0.9337
CCL4 (pg/ml)	3457 ± 1639	4357 ± 2254	0.7783
FABP4 (pg/ml)	4180 ± 509.0	3479 ± 349.7	0.2568
IL1α (pg/ml)	43843 ± 7747	54343 ± 9283	0.1553
IL6 (pg/ml)	45523442 ± 11284336	49899692 ± 11125893	0.1896
IL10 (pg/ml)	222.8 ± 59.36	139.3 ± 69.15	0.1943
IL17 (pg/ml)	12124 ± 1174	18124 ± 2174	0.0086
Leptin (pg/ml)	0	0	-
MMP2 (pg/ml)	268642 ± 38883	375696 ± 47330	0.0049
MMP9 (pg/ml)	15595 ± 1853	15235 ± 1560	0.8977
OPN (pg/ml)	695.3 ± 310.4	548.2 ± 192.3	0.3154
Thrombospondin (pg/ml)	38211 ± 12975	56742 ± 19490	0.0711
CCL2 (pg/ml)	29758667 ± 9344179	26952846 ± 9499983	0.7027
COL1A1 (pg/ml)	467619 ± 98071	946051 ± 215781	0.0141
IFNγ (pg/ml)	19736 ± 7152	10820 ± 1550	0.2825
IL1β (pg/ml)	120949 ± 13975	128866 ± 21131	0.4225
IL8 (pg/ml)	32803 ± 6984	45239 ± 7876	0.0478
IL11 (pg/ml)	1927 ± 411.8	3229 ± 1223	0.2204
IL6R (pg/ml)	337.5 ± 337.5	4400 ± 2787	0.2217
MMP1 (pg/ml)	1.371e ⁰⁰⁶ ± 437329	1.785e ⁰⁰⁶ ± 518468	0.0491
MMP8 (pg/ml)	40201 ± 11637	81293 ± 15457	0.0136
MMP13 (pg/ml)	6847 ± 651.0	6250 ± 364.7	0.4669
Resistin (pg/ml)	1583420 ± 1526729	62706 ± 3318	0.3643
TNFα (pg/ml)	62840 ± 16243	100800 ± 10825	0.1202

Supplementary table S5. Continuous variables of experiments performed.

Fig 1 HFP EF-like syndrome cardiac biopsies exhibit endogenous DNA damage and DDR activation						
		HC	HFP EF	p	n (HC)	n (HFP EF)
IF	% γH2AX ⁺ / total nuclei	1.23±0.41	13.87±5.71	0.05	7	7
IF	% of CD44 ⁺ γH2AX ⁺ / CD44 ⁺ nuclei	1.79±1.79	17.55±5.45	0.02	7	7
IF	% of TNT- γH2AX ⁺ / total nuclei	0.70±0.36	11.43±13.83	0.06	7	7
		TNT-	TNT+		n (HFP EF)	n (HFP EF)
IF	% γH2AX ⁺ cells	63.72±13.24	36.28±13.24		7	7
		HC	HFP EF	p	n (HC)	n (HFP EF)
WB	γH2AX/GAPDH	1.00±0.24	1.97±0.26	0.03	4	5
WB	pCHK1/GAPDH	1.00±0.35	3.93±0.80	0.02	4	5
WB	pCHK2/GAPDH	1.00±0.33	1.85±0.19	0.05	4	5
Fig 2 C-MSC are involved in HFP EF-like syndrome pathogenesis						
		HC	HFP EF	p	n (HC)	n (HFP EF)
WB	γH2AX/GAPDH	1.00±0.49	5.43±1.25	0.03	3	3
WB	pCHK1/GAPDH	1.00±0.35	3.74±0.89	0.04	3	3
WB	pCHK2/GAPDH	1.00±0.49	5.85±1.53	0.04	3	3
RT PCR	IL1β/GAPDH	1.00±0.97	7.00±2.17	0.04	4	4
RT PCR	IL6/GAPDH	1.00±0.97	7.41±2.40	0.05	4	4
RT PCR	TNFα/GAPDH	1.00±0.43	32.38±9.85	0.04	4	4
RT PCR	NF-kB/GAPDH	1.00±0.37	2.17±0.18	0.03	4	4
RT PCR	TGFβ/GAPDH	1.00±0.20	2.88±0.60	0.03	4	4
Fig 3 Stretch-induced mechanical stress activates the DDR in HC C-MSCs						
		Non stretched	Stretched	p	n (NS)	n (S)
	Roundness	0.65±0.02	0.61±0.02	0.008	6	6
	% γH2AX ⁺	10.30±4.50	50.77±11.29	0.02	6	6
WB	γH2AX/GAPDH	1.00±0.26	2.88±0.94	0.05	6	6
WB	pCHK1/GAPDH	1.00±0.50	1.87±0.69	0.03	6	6
WB	pCHK2/GAPDH	1.00±0.54	1.11±0.50	0.44	6	6
WB	αSMA/GAPDH	1.00±0.41	2.63±0.67	0.03	6	6
WB	TGFβ/GAPDH	1.00±0.39	1.52±0.46	0.025	6	6
Fig 4 Cytokines released in the culture supernatant by control C-MSC in static or dynamic culture conditions						
	See Supplementay Table S4					
Fig 5 DDR inhibitor AZ20 prevents pro-fibrotic commitment						
		Stretched	Stretched+ AZ20	p	n (S)	n (S+AZ20)
WB	pCHK1/GAPDH	1.00±0.11	0.76±0.13	0.01	3	3

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WB	γ H2AX/GAPDH	1.00±0.043	0.72±0.033	0.04	3	3
WB	α SMA/GAPDH	1.00±0.14	0.76±0.17	0.02	3	3
WB	TGF β /GAPDH	1.00±0.03	0.80±0.05	0.02	3	3
WB	COL1A1/GAPDH	1.00±0.24	0.69±0.25	0.03	3	3

Fig S1 HFrEF-like syndrome patients cardiac tissue characterization

		HC	HFrEF	p	n (HC)	n (HFrEF)
WB	alfa SMA	1.00±0.12	3.21±0.19	<0.0001	7	7
Masson Trichr	fibrotic area/tissue area (%)	2.71±0.52	16.93±5.87	0.04	7	7
Tunel assay	positive nuclei/total nuclei (%)	0.86±0.17	6.59±2.35	0.0205	5	5
IF	CM area [μ m ²]	96.79±26.59	11450±1293	0.0001	4	4

Fig S2 ATM inhibitor AZD0156 has no effect on pro-fibrotic commitment

		Stretched	Stretched +AZD0156	p	n (S)	n (S+AZD0156)
WB	γ H2AX/GAPDH	1.00±0.33	1.11±0.51	0.43	3	3
WB	pCHK2/GAPDH	1.00±0.60	0.27±0.04	0.35	3	3
WB	α SMA/GAPDH	1.00±0.31	1.46±0.81	0.46	3	3

Fig S3 Senescence in C-MSC derived from HC and HFrEF-like syndrome

		HC	HFrEF	p	n (HC)	n (HFrEF)
Beta Gal	% cells positive for beta gal	6.04±0.79	5.18±1.15	0.55	7	7

Supplementary Figure S1: Verification of the cardiac tissue defects in HFpEF-like syndrome patients.

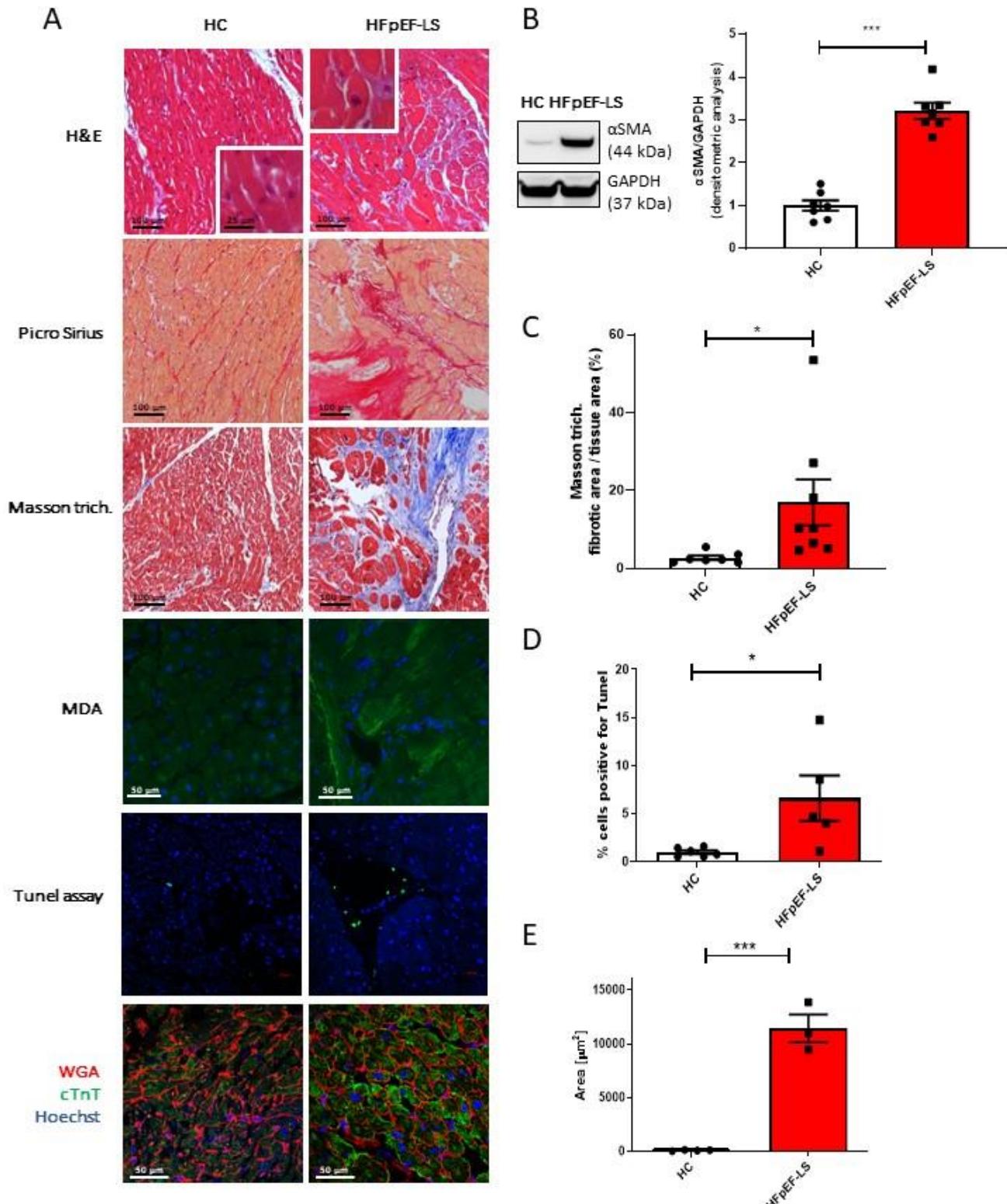
A) HFpEF-like syndrome cardiac tissue is characterized by enlarged nuclei and hypertrophic cardiac fibers (Hematoxylin and Eosin staining; cTnT and WGA immunofluorescence), higher collagen deposition (Picro Sirius and Masson Trichrome staining), higher oxidative stress (malondialdehyde immunofluorescence) and apoptosis (Tunel assay), if compared to HC cardiac tissue.

B) Western blots of α SMA from total protein extract of HC and HFpEF-like syndrome cardiac tissue. Immunostaining of the housekeeping GAPDH is shown for normalization. Densitometric analyses of α SMA levels, normalized on GAPDH. *** p<0.001.

C) Automated computer-based immunofluorescence image analysis of fibrotic tissue (blue) on total tissue area of Masson Trichrome stained cardiac tissues.

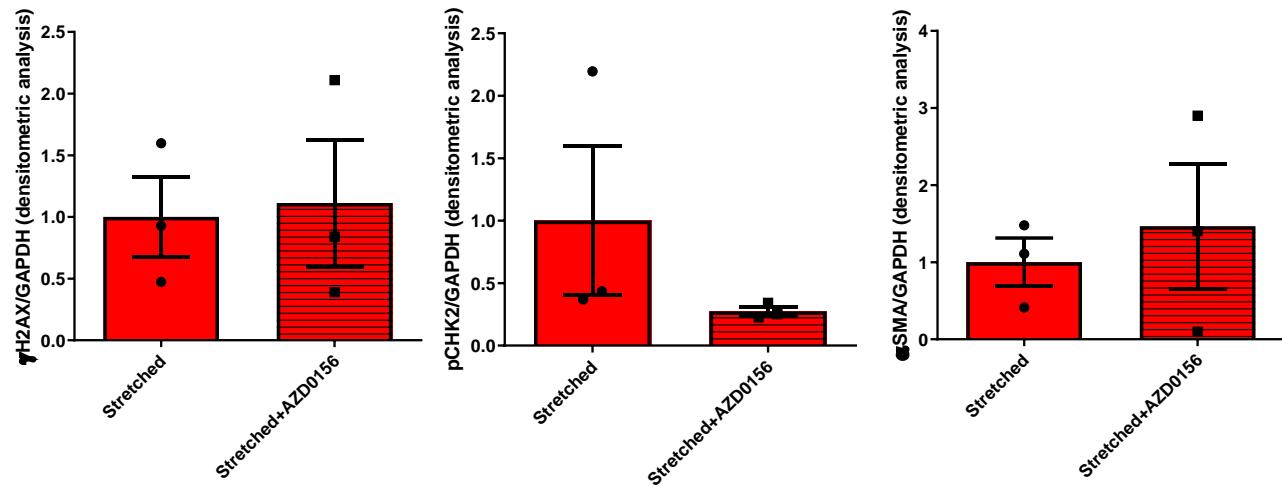
D) Quantification of the percentage of cells positive for Tunel in the cardiac tissue.

E) Quantification of cardiomyocyte hypertrophy confirms the larger dimension of HFpEF-like syndrome cardiomyocytes than HC.



Supplementary Figure S2. ATM inhibitor AZD0156 has no effect on pro-fibrotic commitment

Densitometric analyses of Western blot of proteins extracted from stretched HC C-MSC with/out treatment with 0.5 mM AZD0156, and immunoassayed for γ H2AX, pCHK2, and α SMA. Protein levels, normalized on GAPDH are shown.



Supplementary Figure S3. Senescence in C-MSC isolated from HC and HFpEF-like syndrome samples.

Quantification of the percentage of cells positive for β galactosidase in HC and HFpEF-like syndrome C-MSC.

